HHS Public Access

Author manuscript

J Med Entomol. Author manuscript; available in PMC 2019 May 04.

Published in final edited form as:

J Med Entomol. 2018 May 04; 55(3): 739–741. doi:10.1093/jme/tjx248.

Lack of Evidence for Transovarial Transmission of the Lyme Disease Spirochete *Borrelia mayonii* by Infected Female *Ixodes scapularis* (Acari: Ixodidae) Ticks

Nicole E. Breuner, **Andrias Hojgaard**, and **Lars Eisen**[1]

Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521

Abstract

The recently described Lyme disease spirochete *Borrelia mayonii* is associated with human illness in the Upper Midwest of the United States. Experimental laboratory studies and field observations on natural infection indicate that *B. mayonii* is maintained by horizontal transmission between tick vectors and vertebrate reservoirs. While maintaining a colony of *Ixodes scapularis* Say ticks infected with the *B. mayonii* type strain (MN14-1420), we had an opportunity to examine whether infected females may pass this spirochete transovarially to their offspring. We found no evidence of *B. mayonii* infection in subsets of larvae originating from 18 infected *I. scapularis* females (grand total of 810 larvae tested), or in mice exposed to larval feeding.

Keywords

Borrelia mayonii; *Ixodes scapularis*; Lyme disease; transovarial transmission

Borrelia mayonii, a recently described human-pathogenic species within the *Borrelia burgdorferi* sensu lato complex, is associated with Lyme disease in the Upper Midwest of the United States (Pritt et al. 2016a, b; Kingry et al. 2016). The blacklegged tick, *Ixodes scapularis* Say, is an experimentally confirmed vector of *B. mayonii* (Dolan et al. 2016, 2017a), and natural infection was documented from *I. scapularis* nymphs and adults in Minnesota and Wisconsin (Pritt et al. 2016a, b). Moreover, the house mouse (*Mus musculus* L.) is an experimental reservoir for *B. mayonii* (Dolan et al. 2016, 2017b), and natural infection was documented in Minnesota from two rodent species, the white-footed mouse (*Peromyscus leucopus* Rafinesque) and the American red squirrel (*Tamiasciurus hudsonicus* Erxleben) (Johnson et al. 2017). These findings collectively indicate that *B. mayonii* is maintained in part by horizontal transmission between tick vectors and vertebrate hosts, similar to *Borrelia burgdorferi* sensu stricto (Piesman and Gern 2004). However, the potential for transovarial (vertical) transmission of *B. mayonii* from infected females to their offspring, which could place humans at risk for bites by infected larval ticks, is unknown.

[1]Corresponding author, evp4@cdc.gov.

Disclaimer

The findings and conclusions of this study are by the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Experimental evidence of transovarial transmission is lacking for *I. scapularis* females infected with well characterized *B. burgdorferi* sensu stricto strains. Wild spirochete strains detected by microscopy or immunofluorescence assays from unfed *Ixodes* larvae in older studies can no longer be assumed to belong to *B. burgdorferi* sensu lato (Richter et al. 2012, Rollend et al. 2013) because these detection methods may not differentiate *B. burgdorferi* sensu lato spirochetes from the relapsing fever spirochete *Borrelia miyamotoi*, which is passed transovarially from infected *I. scapularis* females to the resulting larvae (Scoles et al. 2001, Breuner et al. 2017).

In order to conduct experiments on transmission of *B. mayonii* by feeding ticks, we established a colony of *I. scapularis* ticks infected with the MN14-1420 type strain that was originally isolated from a human patient (Pritt et al. 2016a, b). The maintenance of this colony of *B. mayonii*-infected ticks also allowed us to opportunistically examine whether infected females may pass this recently discovered Lyme disease spirochete transovarially to their offspring.

Materials and Methods

Infected female ticks were produced by feeding nymphs from our in-house CT (Connecticut) and MN (Minnesota) colonies infected with the *B. mayonii* type strain (MN14-1420) (Eisen et al. 2017) ad libitum on naïve 1–3 mo old female CD-1 white mice (Charles River Laboratories, Wilmington, MA) as described previously (Dolan et al. 2016). The nymphs had acquired *B. mayonii* by feeding as larvae on 4 individual infected mice from which other resulting nymphs had infection rates ranging from 5.4–20.0% (Dolan et al. 2016, Eisen et al. 2017). Mixed potentially infected females from the CT and MN colonies were then fed together with male ticks on a single female New Zealand white rabbit (Charles River Laboratories). The ticks were allowed to attach to the ears of the rabbit, contained within cloth bags covering each ear and secured with athletic tape at the base of the ears (Piesman et al. 1991, 1999), with each ear receiving approximately 25 female and 25 male ticks. The rabbit was fitted with an Elizabethan collar to prevent disruption and damage to the ear bags and the ticks within. Recovered fully fed females (n=39) were placed singly into glass vials with a plaster of Paris and activated charcoal mixture at the bottom and a mesh top, and then transferred to desiccators (90–95% relative humidity) in a growth chamber maintained at 24°C with a 16:8 hour light:dark cycle.

Whole bodies of fed females that had finished ovipositing (n=23) were examined for presence of *B. mayonii* DNA by multiplex polymerase chain reaction (PCR) as described previously (Dolan et al. 2017, Johnson et al. 2017). Larvae originating from females found to be infected (n=18) were similarly examined for presence of *B. mayonii* DNA by multiplex PCR. This included 45 larvae per infected female, tested in pools of 15 larvae. Moreover, larvae originating from 3 *B. mayonii*-infected females were allowed to feed ad libitum on 10 CD-1 mice (>100 larvae per mouse). These mice were assayed, as described previously (Dolan et al. 2017a, Eisen et al. 2017), for *B. mayonii* infection by culture of ear biopsies (taken 4 wk after the larval feed) in modified Barbour-Stoenner-Kelly medium with antibiotics (in-house BSK-R medium), and for seroreactivity to *B. mayonii* (8 wk after the larval feed).

Animal use and experimental procedures were in accordance with approved protocols on file with the Centers for Disease Control and Prevention Division of Vector-Borne Diseases Animal Care and Use Committee.

Results and Discussion

Of the 23 *I. scapularis* females that fed and oviposited, 18 (78%) contained *B. mayonii* DNA. This infection rate was much higher than expected from previous examination of unfed nymphs of the same cohort (5–20%) that originated from larval feeds on the same 4 individual infectious source animals as the females examined here. Indeed, the observed infection rate of 78% (18/23) for the fed females is significantly higher than an upper end prediction of 20% infected unfed females based on the data for the preceding nymphal stage (Likelihood Ratio Test; $P<0.001$). Co-feeding transmission where spirochetes were passed from feeding infected to non-infected females is one possible explanation for the observed rise in *B. mayonii* infection rate of the fully fed females. Co-feeding transmission from infected to non-infected *I. scapularis* ticks was previously observed for uncharacterized *B. burgdorferi* sensu lato spirochetes in females fed together within ear bags on naïve rabbits, with half of previously uninfected females acquiring spirochetes via co-feeding transmission (Piesman et al. 1998). Moreover, co-feeding transmission from infected to non-infected *I. scapularis* ticks was documented for *B. burgdorferi* sensu stricto (B31) and uncharacterized *B. burgdorferi* sensu lato spirochetes in immatures fed in close proximity to each other on rodents (Patrican 1997, Piesman and Happ 2001). However, as we did not test the blood of the rabbit the females were feeding on for presence of *B. mayonii* in the present study, we cannot rule out the possibility that the females acquired spirochetes via infected blood rather than by co-feeding transmission.

Regardless of whether they were infected prior to feeding or became infected while feeding, we found no evidence that any of the 18 *B. mayonii*-infected *I. scapularis* females passed spirochetes to the resulting larvae (based on testing of a grand total of 810 larvae). As we cannot be sure of how many females were infected prior to feeding, and we tested only subsets of the resulting larvae, the possibility of ineffective transovarial transmission resulting in occasional larval infection cannot be ruled out. Moreover, none of 10 mice exposed to larval feeding were found to be either infected with or seroreactive to *B. mayonii*. We conclude that while horizontal transmission between vector ticks and vertebrate reservoirs appear to be important for the natural maintenance of *B. mayonii* evidence for transovarial transmission of this Lyme disease spirochete is lacking.

Acknowledgments

We thank Marc Dolan, Geoff Lynn, Robert Prose, and Adam Replogle of the Centers for Disease Control and Prevention for technical assistance, and Dr. Neeta Connally of Western Connecticut State University and Dr. Jenna Bjork of the Minnesota Department of Health for providing source material to start tick colonies.

References Cited

Breuner NE, Dolan MC, Replogle AJ, Sexton C, Hojgaard A, Boegler KA, Clark RJ, Eisen L. Transmission of *Borrelia miyamotoi* sensu lato relapsing fever group spirochetes in relation to

duration of attachment by *Ixodes scapularis* nymphs. Ticks Tick Borne Dis. 2017; 8:677–681. [PubMed: 28501504]

Dolan MC, Hojgaard A, Hoxmeier JC, Replogle AJ, Respicio-Kingry LB, Sexton C, Williams MA, Pritt BS, Schriefer ME, Eisen L. Vector competence of the blacklegged tick, *Ixodes scapularis*, for the recently recognized Lyme borreliosis spirochete Candidatus *Borrelia mayonii*. Ticks Tick Borne Dis. 2016; 7:665–669. [PubMed: 26922324]

Dolan MC, Breuner NE, Hojgaard A, Boegler KA, Hoxmeier JC, Replogle AJ, Eisen L. Transmission of the Lyme disease spirochete *Borrelia mayonii* in relation to duration of attachment by nymphal *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol. 2017a; :54.doi: 10.1093/jme/tjx089

Dolan MC, Breuner NE, Hojgaard A, Hoxmeier JC, Pilagard MA, Replogle AJ, Eisen L. Duration of *Borrelia mayonii* infectivity in an experimental mouse model for feeding *Ixodes scapularis* larvae. Ticks Tick Borne Dis. 2017b; 8:196–200. [PubMed: 27838290]

Eisen L, Breuner NE, Hojgaard A, Hoxmeier JC, Pilgard MA, Replogle AJ, Biggerstaff BJ, Dolan MC. Comparison of vector efficiency of *Ixodes scapularis* (Acari: Ixodidae) from the Northeast and Upper Midwest of the United States for the Lyme disease spirochete *Borrelia mayonii*. J Med Entomol. 2017; 54:239–242. [PubMed: 28082653]

Johnson TL, Graham CB, Hojgaard A, Breuner NE, Maes SE, Boegler KA, Replogle AJ, Kingry LC, Petersen JM, Eisen L, Eisen RJ. Isolation of the Lyme disease spirochete *Borrelia mayonii* from naturally infected rodents in Minnesota. J Med Entomol. 2017; 54:1088–1092. [PubMed: 28444198]

Kingry LC, Batra D, Replogle A, Rowe LA, Pritt BS, Petersen JM. Whole genome sequence and comparative genomics of the novel Lyme borreliosis causing pathogen, *Borrelia mayonii*. PLoS ONE. 2016; 11:e0168994. [PubMed: 28030649]

Patrican LA. Acquisition of Lyme disease spirochetes by cofeeding *Ixodes scapularis* ticks. Am J Trop Med Hyg. 1997; 57:589–593. [PubMed: 9392600]

Piesman J, Gern L. Lyme borreliosis in Europe and North America. Parasitology. 2004; 129:S191–S220. [PubMed: 15938512]

Piesman J, Happ CM. The efficacy of co-feeding as a means of maintaining *Borrelia burgdorferi*: A North American model system. J Vector Ecol. 2001; 26:216–220. [PubMed: 11813659]

Piesman J, Maupin GO, Campos EG, Happ CM. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi* with description of a needle aspiration isolation method. J Infect Dis. 1991; 163:895–897. [PubMed: 2010643]

Piesman, J., Dolan, MC., Burkot, TR., Schriefer, ME. Ability of three person-biting tick species from the eastern United States to transmit *Borrelia burgdorferi*. In: Coons, L., Rothschild, M., editors. The Second International Conference on Tick-Borne Pathogens at the Host-Vector Interface: A Global Perspective, The Conference. 1998. p. 206-211.

Piesman J, Clark KL, Dolan MC, Happ CM, Burkot TR. Geographic survey of vector ticks (*Ixodes scapularis* and *Ixodes pacificus*) for infection with the Lyme disease spirochete, *Borrelia burgdorferi*. J Vector Ecol. 1999; 24:91–98.s. [PubMed: 10436883]

Pritt BS, Mead PS, Johnson DKH, Nietzel DF, Respicio-Kingry LB, Davis JP, Schiffman E, Sloan LM, Schriefer ME, Replogle AJ, Paskewitz SM, Ray JA, Bjork J, Steward CR, Deedon A, Lee X, Kingry LC, Miller TK, Feist MA, Theel ES, Patel R, Irish CL, Peterson JM. Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. Lancet Infect Dis. 2016a; 16:556–564. [PubMed: 26856777]

Pritt BS, Respicio-Kingry LB, Sloan LM, Schriefer ME, Replogle AJ, Bjork J, Liu G, Kingry LC, Mead PS, Neitzel DF, Schiffman E, Johnson DKH, Davis JP, Paskewitz SM, Boxrud D, Deedon A, Lee X, Miller TK, Feist MA, Steward CR, Theel ES, Patel R, Irish CL, Peterson JM. *Borrelia mayonii* sp nov., a member of the *Borrelia burgdorferi* sensu lato complex, detected in patients and ticks in the upper Midwestern United States. Int J Sys Evol Microbiol. 2016b; 66:4878–4880.

Richter D, Debski A, Hubalek Z, Matuschka F. Absence of Lyme disease spirochetes in larval *Ixodes ricinus* ticks. Vector Borne Zoonotic Dis. 2012; 12:21–27. [PubMed: 21923267]

Rollend L, Fish D, Childs JE. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: A summary of the literature and recent observations. Ticks Tick Borne Dis. 2013; 4:46–51. [PubMed: 23238242]

Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 2001; 1:21–34. [PubMed: 12653133]